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Factors associated with recurrence and survival length following relapse in patients with neuroblastoma

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Running title: Survival in patients with relapsed neuroblastoma

Abstract

Purpose: Despite therapeutic advances, survival following relapse for neuroblastoma patients remains poor. We investigated clinical and biological factors associated with length of progression free and overall survival following relapse in UK neuroblastoma patients.

Methods: All cases of relapsed neuroblastoma, diagnosed 1990-2010, were identified from four Paediatric Oncology principal treatment centres. Kaplan-Meier and Cox regression analyses were used to calculate post relapse overall survival (PROS), post relapse progression free survival (PRPFS) between relapse and further progression, and to investigate influencing factors.

Results: 189 cases were identified from case notes, 159 (84.0%) high risk and 17 (9.0%), unresectable, *MYCN* non-amplified (non-MNA) intermediate risk (IR). For high risk patients diagnosed >2000, median PROS was 8.4 months (inter quartile range (IQR) = 3.0-17.4) and median PRPFS was 4.7 months (IQR=2.1-7.1). For IR, unresectable non-MNA patients, median PROS was 11.8 months (IQR 9.0-51.6) and 5-year PROS was 24% (95% CI 7%-45). *MYCN* amplified (MNA) disease and bone marrow metastases at diagnosis were independently associated with worse PROS for high risk cases. 80% of high risk relapses occurred within 2 years of diagnosis compared with 50% of unresectable non-MNA IR disease.

Conclusion: Patients with relapsed HR neuroblastomas should be treatment stratified according to *MYCN* status and PRPFS should be the primary endpoint in early phase clinical trials. The failure to salvage the majority of IR neuroblastoma is concerning, supporting investigation of intensification of

upfront treatment regimens in this group to determine whether their use would diminish likelihood of relapse.

Keywords: relapsed neuroblastoma, epidemiology, post relapse progression free survival, post relapse overall survival, high risk, intermediate risk

Introduction

Neuroblastoma, the second most common childhood solid tumour, accounts for 8% of all childhood (0-14 years) cancers in the UK (Stiller, 2007). It is one of the most difficult childhood cancers to cure with UK & Ireland five-year survival of 64.7% for cases diagnosed during 2005-2007 (Gatta *et al*, 2014).

However, survival remains poor for children diagnosed with high-risk disease (50% of all neuroblastoma) (Cohn *et al*, 2009) defined as stage 4 >1 year of age, or *MYCN* amplified (MNA) localised (stage 2 & 3) or MNA infant (<12 month) disease, with relapse in >50% of cases (Maris *et al*, 2007; Maris, 2010). Relapse also occurs in other risk groups including intermediate risk (around 20% of cases at diagnosis) defined as *MYCN* non-amplified (non-MNA), unresectable (stage 3) and non-MNA stage 4 <12 months old cases. The presence of MNA is a well-established poor prognostic marker in patients with neuroblastoma with localized disease and infants <12 months of age (Cohn *et al*, 2004; Maris *et al*, 2007; Canete *et al*, 2009). Survival from relapsed high risk neuroblastoma is currently less than 10% (London *et al*, 2011; Park *et al*, 2013). The disease control intervals and the patterns of recurrence are important for evaluation of new treatment strategies and early phase study designs, as they are increasingly being used to define alternative end-points to tumour response (Santana *et al*, 2008; Fox *et al*, 2014).

Some recent studies have reported clinical features of relapsed neuroblastoma (Garaventa *et al*, 2009; London *et al*, 2011; Simon *et al*, 2011), however few report length of post-relapse progression free survival. The present study aimed to investigate factors associated with recurrence, survival

length following relapse and length of progression free survival in patients with neuroblastoma diagnosed and treated at four UK Paediatric Oncology principal treatment centres. This study included information from patient file review on post-relapse progression free survival, which is essential for informing the design and judging the efficacy of new treatments tested in early phase clinical trials.

Methods

Study Patients

All cases of relapsed and refractory/progressive neuroblastoma diagnosed 1990–2010, were identified from four UK Paediatric Oncology principal treatment centres (The Royal Victoria Infirmary, Newcastle, Leeds Teaching Hospitals NHS Trust, The Royal Manchester Children's Hospital and The Royal Marsden Hospital). Population-based data from three specialist registries were used: the Northern Region Young Persons' Malignant Disease Registry (NRYPMDR) (Cotterill *et al*, 2000), the Yorkshire Specialist Register of Cancer in Children and Young People (YSRCCYP) (Feltbower *et al*, 2004) and the Manchester Children's Tumour Registry (MCTR) (Birch, 1988), and a non-population database held at the Royal Marsden Hospital NHS Foundation Trust. All cases within the age ranges of the registries were included. 1990 was chosen as the start date as from this date onwards combination chemotherapy and high dose myeloablative therapy were routinely used to treat high risk neuroblastoma (Pritchard *et al*, 2005; Pearson *et al*, 2008). Nine patients who died within seven days of relapse were excluded as their survival and treatment data at relapse would be limited.

Definition of Relapse

The International Neuroblastoma Response Classification (INRC) criteria were used to define relapse as a new site of disease or 25% increase in tumour size following an initial response (including partial) to treatment, and refractory disease as tumours which did not respond to any first, second or third line therapies and subsequently progressed (Brodeur *et al*, 1993).

Statistical Analysis

Table 1 and Supplementary Table S1 show the demographic and clinical variables studied at diagnosis and relapse. Post-relapse overall survival time (PROS) was the primary end point, defined as time from first relapse/progression (including relapsed refractory disease) to death or date of last follow-up in survivors. Follow-up was censored at 31st March 2014. Kaplan-Meier methods were used to calculate estimates of PROS and post-relapse progression free survival (PRPFS) which is the time between relapse and further relapse/progression (Kaplan & Meier, 1958). Log-rank tests were used to compare differences in survival estimates between variables. Cox proportional hazards regression analysis was used to investigate risk factors that may influence PROS (Cox, 1972). The associations between PROS and *MYCN* amplification, 1p deletion, time interval from diagnosis to relapse and age at diagnosis were analysed. Age at diagnosis was categorised into <18 months, ≥18 months and < 5 years, ≥ 5 years, since 18 months is now used to stratify patients at diagnosis and there is evidence that older children have a more protracted relapse course (Cohn *et al*, 2009). Time interval from diagnosis to relapse was categorised as <6 months, 6 to <12 months, 12 to <18 months, 18 to <24 months and ≥24 months to enable comparison with previously published studies (London *et al*, 2011; Simon *et al*, 2011). Two time periods of diagnosis, ≤ 2000 and > 2000, were used to categorise patients as around this time treatment for high risk neuroblastoma was intensified (Kohler *et al*, 2007; Pearson *et al*, 2008; Ladenstein *et al*, 2010; Kohler *et al*, 2013). Only models that met the Cox proportional hazard assumption are presented (Cox, 1972).

Subgroup analyses by risk group were carried out for high risk and intermediate risk (IR) patients using the International Neuroblastoma Risk

Group (INRG) definitions and a comparison of survivors versus non-survivors (Cohn *et al*, 2009). Detailed analysis of unresectable, non-MNA cases was undertaken as this group has varied clinical behaviour (Park *et al*, 2009; Baker *et al*, 2010). Where the total number of cases was low, the 95% Wilson confidence intervals for binomial percentages were calculated. Stata version 12 was used for all analyses, with statistical significance taken to be $P<0.05$ (StataCorp, 2011).

Results

189 cases of relapsed neuroblastoma, were identified in this study. A flow diagram showing numbers of patients by risk group is given in Figure 1. For all relapsed cases PROS significantly increased after 2000 (P<0.001) (Supplementary Figure S1a and Supplementary Figure S1b).

High risk group (Table 1 & Supplementary Table S1)

The number of cases analysed for each variable differed depending on the completeness of available data. At diagnosis, 90% of cases were treated in a clinical trial or per clinical trial protocol (Supplementary Table S2). The overall response assessment according to the INRC criteria (Brodeur *et al*, 1993) at the end of first line treatment was: partial response including very good partial response for 70/139 (50%) cases and complete response for 30/139 (22%) cases. At first relapse, 124/159 (78%) of cases relapsed within 2 years of diagnosis and 13/107 (12%) of patients relapsed at the primary site alone. In 60 patients (38%) in whom levels of urinary catecholamines were recorded at diagnosis and relapse, 48 (80%) had raised urinary catecholamines levels at both diagnosis and relapse.

The median PROS time for all high risk cases was 4.5 months (inter-quartile range (IQR) 1.9–11.4) which was significantly increased for patients diagnosed after 2000 versus before (P<0.001) (Figure 2a, Figure 2b, Supplementary Figure S2a and Supplementary Figure S2b). Significantly more patients diagnosed after 2000, 60/74 (81%), were treated actively at relapse compared to those who were given palliative radiotherapy or supportive care alone 14/74 (19%); versus 22/64 (44%) ≤2000) (P=0.03). Active treatments at relapse included second line chemotherapy, mIBG therapy, or second line

chemotherapy followed by other treatments such as radiotherapy, or phase I or II clinical trials in 10/138 (7%) cases (Table 2). Figure 3 shows that for cases diagnosed after 2000, 6/76 (8%) patients treated by mIBG therapy had a median PROS of 12.2 months (IQR = 10.9-26.2 months), 5/76 (7%) patients enrolled onto phase I or II trials had a median PROS of 13.5 months (IQR = 7.7–16.4) and 29/76 (38%) treated by second line chemotherapy had a median PROS of 6.6 months (IQR = 2.5-17.5) compared to 1.5 months (IQR = 0.6-2.9) for cases treated by supportive care (*P*=0.01). In contrast, before 2000 only one patient had mIGB therapy. For 58 cases, the date of further relapse/progression was recorded and for those patients the median PRPFS was 4.5 months (IQR 2.2–8.7 months); it was 3.9 months (IQR 2.5-8.7 months) for 21 cases diagnosed before 2000 and 4.7 months (IQR 2.1-7.1 months) for 37 cases diagnosed after 2000 (*P*=0.54). Treatments received at subsequent relapse/progression are given (Supplementary Table S3).

Cox univariable proportional hazards regression analyses for high risk cases showed that PROS time was significantly worse for cases with MNA disease (*P*<0.0001), both MNA and 1p deleted disease (*P*=0.02), liver metastases at diagnosis (*P*=0.02), and for cases who relapsed within 6 months of diagnosis compared to relapses >2 years (*P*=0.03), while patients >5 years old at diagnosis had longer PROS (*P*=0.02) (Supplementary Table S4). However, in multivariable analysis only MNA (adjusted HR=2.06, 95% CI 1.22–3.46, *P*=0.007) and bone marrow metastases at diagnosis (adjusted HR=2.33, 95% CI 1.26–4.29, *P*=0.007) were independently significantly associated with worse PROS (Table 3). Information on *MYCN* status was unknown for 30% of cases, so sensitivity analysis was carried out to include the *MYCN* unknown

cases as an additional category in the model which showed that it did not affect the results. MNA disease was significantly associated with worse PROS (*P*<0.001) (Figure 2c and Supplementary Figure 2c). Similar results were obtained for PROS for cases diagnosed before (*P*<0.001) (Figure 2d) or after 2000 (*P*=0.02) (Figure 2e).

Intermediate risk, unresectable, *MYCN* non-amplified group (Table 1 & Supplementary Table S1)

The number of cases analysed for each variable differed depending on the completeness of available data. INPC histology was unfavourable in 13/15 (87%; 95% CI 62-96%) cases and primary tumour site was abdominal in 14/17 (82%; 95% CI 59-94%)12/17 (71%; 95% CI 47-87%) were treated in a clinical trial at diagnosis or per clinical trial protocol (4 cases in European Neuroblastoma Study group Fifth study (ENSG5) (Pearson *et al*, 2008), 2 cases in European High Risk Neuroblastoma Study 1 of SIOP-Europe (HRNBL1) (Ladenstein *et al*, 2010), 2 in unresectable NB2009 (Kohler *et al*, 2013), 4 in ENSG9 (ClinicalTrials.gov identifier: NCT00276731) (Supplementary Table S2). At the end of treatment 7/15 (47%; 95% CI 25-70%) cases had achieved partial response and 6/15 (40%, 95% CI 20-64%) complete response. The median PROS time for this group was 11.8 months (IQR=9.0-51.6) (Supplementary Figure S3a and Supplementary Figure S3b).

Significantly more cases relapsed >2 years from diagnosis 8/17 (47%%; 95% CI 26-69%) compared to high risk cases 35/159 (22%; 95% CI 16-29) (*P*=0.04). 8/13 (62%, 95% CI 36-82%) relapsed at the primary site. In 9/17 patients (53%, 31-74%) in whom levels of urinary catecholamines were recorded at diagnosis and relapse, 3/9 (33%, 95% CI 12-65%) had raised

urinary catecholamine levels at both diagnosis and relapse. 11/16 patients (69%, 95% CI 44-86%) were treated with second line high risk type chemotherapy at first relapse (Table 2). A further relapse/progression date and treatment were recorded for 8 cases, and for those the median PRPFS was 10.1 months (IQR=7.8–12.3 months). 5/8 (63%, 95% CI 31-86%) were treated with second line chemotherapy and the remainder treated by combined chemotherapy and surgery or radiotherapy including mIBG therapy or supportive care (Supplementary Table S3).

Survivors (Supplementary Table S5)

Only 8% of all relapsed patients in our study survived to the end of the study period. 15/16 patients (8%, 95% CI 72-99%) were disease free 5 years from first relapse. 4/5 of the high risk survivors and 1/4 of the IR were diagnosed >2000. The median follow up time from relapse for survivors was 11 years (IQR 6.6-16.5). 8/16 (50%, 95% CI 28-72%) patients were aged ≤18 months at diagnosis, 5/15 (33%, 95% CI 15-58%) had high risk disease at diagnosis, and 12/13 (85%, 95% CI 58-96%) were non-MNA. At relapse, 8/14 (57%, 95% CI 33-79%) survivors were treated with second line chemotherapy, and/or with radiotherapy and surgery and 1/14 was treated with chemotherapy and autologous stem cell transplant.

Comparing survivors with non-survivors showed that 27% (95% CI 11-52%) of survivors had favourable INPC histology compared with 4% (95% CI 1-8%) of non-survivors (*P*=0.006), 93% (95% CI 70-99%) of survivors had complete or partial overall response at end of treatment compared to 71% (95% CI 64-79%) (*P*=0.04), and 50% relapsed >2 years from diagnosis compared to 21% (95% CI 15-28) of non-survivors (*P*=0.01) (Supplementary Table S6). Of

the high risk survivors, 3/5 were ≥18 months at diagnosis, 4/5 relapsed after initial myeloablative therapy and 1/5 relapsed after surgery.

Discussion

This study was designed to gain knowledge about relapsed neuroblastoma from primary sources of information and to reflect the outcome of these patients, avoiding the selection bias of clinical trials. It is the first multi-institutional study to report on PRPFS which is a very useful parameter for judging the efficacy of early phase clinical trials. The observed interval between relapses is important for early phase trials design as it provides a baseline comparator for exploratory studies of new agents in relapsed neuroblastoma, where the time interval of PRPFS can be used as a primary endpoint when designing exploratory trials (Santana *et al*, 2008 & Fox *et al*, 2014).

The current study reports the demographics of relapsed neuroblastoma cases diagnosed 1990–2010 from four UK Paediatric Oncology principal treatment centres treating around 30% of all UK patients in total. PROS time remains very poor for both high risk and IR (unresectable, non-MNA) disease. The median PROS for high risk cases was 4.5 months with only 7% of relapsed cases surviving more than 5 years. However, PROS has significantly increased for high risk cases diagnosed after the year 2000 compared to before 2000 from 2.9 months to 8.4 months, achieving a 5 year survival of 12.7%. This significant improvement in PROS after 2000 may be due to more patients being treated actively at relapse with second line chemotherapy such as temozolomide, topotecan, irinotecan or oral etoposide. All these chemotherapy regimens increase the PRPFS, but long-term overall survival remains poor (Rubie *et al*, 2006; London *et al*, 2010; Bagatell *et al*, 2011; Fox *et al*, 2014).

High Risk

In contrast to most previous epidemiological studies of relapsed neuroblastoma, our study reports second relapses. The duration from first to subsequent relapse is important particularly for early phase clinical trial design. For a sub-group of patients, the date of a second event was recorded and the median PRPFS time to second event was 4.5 months (IQR=2.2-8.7). This is shorter than that reported by Santana and colleagues who found a median disease progression free interval of 7.2 months between first and second relapse (Santana *et al*, 2008). This may reflect a difference in treatments given, as very few patients in our cohort were treated on phase I or II clinical trials at first or subsequent relapse, partly due to a lack of available applicable trials prior to 2000 (Moreno *et al*, 2013). Recently trials for relapsed neuroblastoma have opened across Europe, such as the BEACON study, a randomised phase II trial exploring the use of bevacizumab and temozolomide or temozolomide-irinotecan (ClinicalTrials.gov identifier: NCT02308527). The results of our study demonstrating the very poor outcomes following relapse strongly argue for the inclusion of patients with high risk relapsed neuroblastoma in randomised clinical trials at relapse whenever possible. This study does not identify a specific group of patients who are less likely to benefit from further treatment at relapse.

Our results for PROS concur with those published using data from the INRG database (2,226 relapsed cases), diagnosed 1990 – 2002, where 5 year PROS for stage 4 cases, >18 months with non-MNA disease was 8%, and only 4% for stage 4 with MNA disease (London *et al*, 2011). Around 55/189 (29%) cases from the present study may overlap with the INRG database. A study from the Italian neuroblastoma registry reported 10 year PROS for stage 4

disease at diagnosis was 1.5% following progression and 2% following relapse (Garaventa *et al*, 2009). Analysis of relapsed high risk neuroblastoma patients from German trials diagnosed 1990-2007, treated with second-line chemotherapy at relapse, found 3-year PROS was 9.6% (Simon *et al*, 2011).

Several prognostic factors are used in risk stratification for neuroblastoma at diagnosis including age at diagnosis, MNA, presence of segmental chromosomal aberrations, metastatic disease and tumour histology (George *et al*, 2001). These factors may also be important in determining response to treatments at relapse. Age >5 years at diagnosis had a longer PROS consistent with studies showing that older children have a more protracted relapse course (Cohn *et al*, 2009). We also found worse PROS in high risk cases with *MYCN* amplification as previously reported (London *et al*, 2011; Garaventa *et al*, 2009; Simon *et al*, 2011; Lau *et al*, 2004), and with both MNA and chromosome 1p deletion at diagnosis. Chromosome 1p deletion has been shown to be associated with shorter median PROS (London *et al*, 2011; Lau *et al*, 2004), poorer event free survival from diagnosis (EFS) (Maris *et al*, 2001), and to be strongly related to high risk features (Attiyeh *et al*, 2005). Both 1p deletion and 11q loss were independently associated with shorter PRPFS in patients with low and IR disease (Attiyeh *et al*, 2005). However, in the present study insufficient data on 11q or other segmental chromosomal abnormalities precluded formal analysis. Interestingly, liver metastases at diagnosis were also associated with shorter PROS in our study. In previous studies liver metastases were associated with MNA and had a worse prognosis in older children (DuBois *et al*, 1999), unlike infants with non-MNA disease (Kushner *et al*, 2006). 90% of all relapsed cases we studied had primary tumours arising in abdominal sites

and only 8% had thoracic or neck tumours. This is in-keeping with other studies reporting that adrenal tumours are associated with unfavourable clinical and biological characteristics and therefore worse EFS in contrast to thoracic tumours which are associated with favourable characteristics and better EFS (Sung *et al*, 2009; Vo *et al*, 2014). However, we acknowledge the small numbers of cases involved in this study and the missing data in some of the variables means some results should be interpreted with caution.

The duration from diagnosis to first relapse has been shown to be a prognostic factor for PROS (London *et al*, 2011; Garaventa *et al*, 2009; Lau *et al*, 2004). Around 80% of high risk cases in our study relapsed within 2 years from diagnosis, and PROS was significantly shorter when relapse occurred within 6 months of diagnosis. Lau and colleagues found that patients who relapsed either within 6 months from diagnosis or 6 months from stem cell transplant had shorter median PROS (Lau *et al*, 2004). Other studies however, reported recurrence within 6 – 24 months from diagnosis implied worse PROS (London *et al*, 2011; Simon *et al*, 2011). These differences may be due to patient heterogeneity; some studies included only high risk cases (Simon *et al*, 2011), whereas others included all relapsed neuroblastoma (London *et al*, 2011), or different definitions of relapse.

The role of urinary catecholamines as a reliable marker for monitoring neuroblastoma relapse is controversial (Simon *et al*, 2003). They may be negative due to small tumour burden at relapse or decreased production in some previously treated neuroblastomas. In the present study, urinary catecholamines were raised at relapse in 80% of patients who had raised catecholamines at diagnosis, but were only measured in 46% of patients at

relapse. The current study suggests that raised urinary catecholamines are helpful as a confirmatory indicator and a tumour marker of response (Kushner *et al*, 2009).

Intermediate Risk

IR disease comprised 13% of relapsed neuroblastomas in our study, 4% who were IR stage 4, non-MNA <12 months old were not further analysed as this subgroup is usually salvageable at relapse (Maris *et al*, 2007; De Bernardi *et al*, 2009). However, 9% were IR, unresectable, non-MNA with mostly unfavourable INPC histology. The prognostic value of pathology in this group was confirmed in a recent SIOPEN study (Kohler *et al*, 2013). Analysis of stage 3 data from the INRG database showed that patients >18 months with undifferentiated INPC histology and elevated levels of serum ferritin had worse overall survival and EFS (Meany *et al*, 2014). In the absence of *MYCN* amplification other genetic markers in this group may be important risk factors, such as tumour cell ploidy (Baker *et al*, 2010; Bagatell *et al*, 2005), 11q aberrations (Attiyeh *et al*, 2005; Meany *et al*, 2014) or other segmental chromosomal abnormalities (Defferrari *et al*, 2015). The management of this group of patients remains controversial, those with unfavourable histological or biological features are treated with intensive multimodality therapy including myeloablative therapy in the US (Baker *et al*, 2010), but not in the UK or Europe. However, in the present study 6/17 patients (35%; 95% CI 17-59%) were treated in high risk protocols (ENSG5 and HRNBL1) at the physician's discretion due to patient age and/or unknown *MYCN* status. Almost 50% of IR, unresectable, non-MNA neuroblastoma relapsed >2 years from diagnosis including 5/17 (29%; 95% CI 13-53%) patients who had local radiotherapy as

part of their frontline treatment at diagnosis. Salvage therapies at relapse failed in 75% of cases, suggesting the need for intensification of upfront treatment regimens for cases with unfavourable histology at diagnosis (Kohler *et al*, 2013), to determine whether their use would diminish the likelihood of relapse.

Conclusion

In conclusion, this study showed that the PROS for neuroblastoma patients has increased but long term survival remains poor. 80% of high risk relapses occur within 2 years from diagnosis, in contrast to only 50% of IR unresectable, non-MNA neuroblastoma, and although this latter group comprise <10% of all relapsed neuroblastoma, the failure to salvage these patients in over 75% of cases, even when given high-risk type treatment at relapse, is concerning. Since MNA disease has a worse OS as highlighted by previous studies, our study underscores the need for early phase clinical trial data to be analysed according to *MYCN* status. Finally, more patients need to be recruited to early phase studies where PRPFS should be considered as the primary endpoint in study design, especially if cytostatic novel therapies are given.

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Table 1: Patient characteristics at relapse for all cases of relapsed neuroblastoma, high risk cases, and intermediate risk, stage 3, unresectable, non-MNA cases.

Table 1:Patient characteristics for all cases of relapsed neuroblastoma, high risk cases and intermediate risk, stage 3, unresectable, non-MNA cases				
	All relapsed neuroblastoma N=189 N (%, 95% CI) [α]	High risk relapsed Neuroblastoma N=159 (84.1%) N (%, 95% CI) [α]	Intermediate risk (unresectable MYCN non-amplified) N=17 (9.0%) N (%, 95% CI) [β]	*P* – value[**] (high vs intermediate risk groups)
Time from diagnosis to relapse				
Mean	18.1 months	17.6 months	28.8 months	
Median	14.7 months	14.6 months	22.1 months	
Inter-quartile range	9.2 – 22.1 months	9.3 – 21.5 months	10.3 – 39.1 months	0.03
Range	1.3 – 96.2 months	1.3 – 96.2 months	4.0 – 68.5 months	
Time from diagnosis to relapse groups				
< 6 months	32 (16.9, 11.9-23.1)	24 (15.1, 9.9-21.6)	1 (5.8, 1.0-27.0)	
6 – 12 months	38 (20.1, 14.6-26.5)	32 (20.1, 14.2-27.2)	4 (23.5, 9.6-47.3)	0.23
12 – 18 months	43 (22.8, 17.0-29.3)	40 (25.2, 18.6-32.6)	2 (11.8, 3.3-34.3)	
18 – 24 months	32 (16.9, 11.9-23.1)	28 (17.9, 12.0-24.4)	2 (11.8, 3.3-34.3)	
>24 months	44 (23.3, 17.5-30.0)	35 (22.0, 15.8-29.3)	8 (47.1, 26.2-69.0)	
Site of relapse	N = 132 (69.8)*	N = 107 (67.3)*	N = 13 (76.5)*	
Primary	30 (22.7, 15.9-30.8)	13 (12.2, 6.6-19.9)	8 (61.5, 35.5-82.3)	
Metastases	102 (77.3, 69.2-84.1)	94 (87.8, 80.1-93.4)	5 (38.5, 17.7-64.5)	<0.001
Missing	57 (30.2) [b]	52 (32.7) [b]	4 (23.5) [b]	
Bone metastases at relapse	N = 170 (89.9)*	N = 142 (75.1)*	15 (88.2)*	
No	87 (51.2, 43.4-58.9)	70 (49.3, 40.8-57.8)	9 (60.0, 35.7-80.2)	
Yes	83 (48.8, 41.1-56.6)	72 (50.7, 42.2-59.2)	6 (40.0, 19.8-64.3)	0.43
Missing	19 (10.1) [b]	17 (24.9) [b]	2 (11.8) [b]	
Bone marrow metastasis at relapse	N = 140 (74.1)*	N = 116 (73.0)*	N = 13 (76.5)*	
No	75 (53.6, 45.0-62.0)	58 (50.0, 40.6-59.4)	9 (69.2, 42.4-87.3)	
Yes	65 (46.4, 38.0-55.0)	58 (50.0, 40.6-59.4)	4 (30.8, 12.7-57.6)	0.24
Missing	49 (25.9) [b]	43 (27.0) [b]	4 (23.5) [b]	
Urinary catecholamine levels at relapse	N = 87 (46.0)*	N = 73 (45.9)*	N = 9 (52.9)*	
Normal	20 (23.0, 14.6-33.2)	14 (19.2, 10.9-30.1)	6 (66.7, 35.4-87.9)	
Elevated	67 (77.0, 66.8-85.4)	59 (80.8, 55.1-77.7)	3 (33.3, 12.1-64-6)	0.006

Missing	102 (54.0) [b]	86 (54.1) [b]	8 (47.1) [b]	
Status				
Alive	16 (8.5, 4.9-13.4)	5 (3.1, 1.0-7.2)	4 (23.5, 9.6-47.3)	
Dead	166 (87.8, 82.3-92.1)	148 (93.1, 88.0-96.5)	13 (76.5, 52.7-90.4)	0.001
Lost to follow-up	7 (3.7, 1.5-7.5)	6 (3.8, 1.4-8.0)	-	

***** Percent of the total number of cases.

** Chi-squared test and the Fisher's exact test (where numbers were less than 5) were used to compare between high and intermediate risk group characteristics. [α] 95% CI were calculated for percentages using the asymptotic method.

[b] Percent with missing data. [β] 95% CI for proportions were calculated using the Wilson score method.

Table 2: Treatment received at first relapse for all relapsed neuroblastoma cases, high risk cases and intermediate risk, stage 3, unresectable, *non-MNA* neuroblastoma.

Table 2: Treatment received at first relapse for all relapsed neuroblastoma cases, high risk cases and intermediate risk, stage 3, unresectable, *non-MNA* neuroblastoma.

Treatment	All relapsed cases N (%)			High Risk N (%)			Intermediate risk N (%)		
	Diagnosed ≤ 2000	Diagnosed > 2000	Total	Diagnosed ≤ 2000	Diagnosed > 2000	Total	Diagnosed ≤ 2000	Diagnosed > 2000	Total
Second line Chemotherapy*	29 (36.3)	33 (38.4)	62 (37.4)	22 (34.4)	29 (39.2)	51 (37.0)	2 (22.2)	1 (14.3)	3 (18.8)
Combination of treatments**	15 (118.8)	17 (19.8)	32 (19.3)	10 (15.6)	12 (16.20	22 (15.9)	4 (44.4)	4 (57.1)	8 (50.0)
mIBG Therapy	1 (1.3)	6 (7.0)	7 (4.2)	1 (1.6)	6 (8.1)	7 (5.1)	0 (0.0)	0 (0.0)	0 (0.0)
Other (e.g. Surgery or radiotherapy)	7 (8.8)	10 (11.6)	17 (10.2)	4 (6.3)	8 (10.8)	12 (8.7)	2 (22.2)	2 (28.6)	4 (25.0)
Phase I or II trials	5 (6.3)	5 (5.8)	10 (6.0)	5 (7.8)	5 (6.8)	10 (7.3)	0 (0.0)	0 (0.0)	0 (0.0)
Palliative radiotherapy	10 (12.5)	6 (7.0)	16 (9.6)	10 (15.6)	6 (8.1)	16 (11.6)	0 (0.0)	0 (0.0)	0 (0.0)
Supportive care	13 (16.3)	9 (10.5)	22 (13.3)	12 (18.8)	8 (10.8)	20 (14.5)	1 (11.1)	0 (0.0)	1 (6.3)
Total	80 (100)	86 (100)	166 (100)	64 (100)	74 (100)	138 (100)	9 (100)	7 (100)	16 (100)

* Second line chemotherapy includes vincristine, temozolamide, irinotecan, topotecan vincristine-doxorubicin or oral etoposide.
** Combination of chemotherapy, surgery, radiotherapy and miBG therapy.

Table 3: Results of multivariable analysis for post-relapse overall survival for high risk cases.

Table 3: Results of multivariable analysis for post-relapse overall survival for high risk cases			
Factor	**N (%)**	**Hazard ratio (95% CI)**	***P*-value**
Age at diagnosis			
≤ 5 years	126 (79.3)	1	
> 5 years	33 (20.7)	0.77 (0.44 – 1.34)	0.35
Bone marrow metastases at diagnosis			
No	26 (17.8)	1	
Yes	120 (82.2)	2.33 (1.26 – 4.29)	0.007
Liver metastases at diagnosis			
No	122 (87.8)	1	
Yes	17 (12.2)	1.44 (0.73 – 2.83)	0.29
***MYCN* disease**			
Not amplified	67 (57.8)	1	
Amplified	49 (42.2)	2.06 (1.22 – 3.46)	0.007
Time from diagnosis to relapse			
>24 months	35 (22.0)	1	
18 – 24 months	28 (17.9)	1.18 (0.58 – 2.40)	0.65
12 – 18 months	40 (25.2)	1.21 (0.65 – 2.24)	0.54
6 – 12 months	32 (20.1)	1.22 (0.65 – 2.31)	0.54
< 6 months	24 (15.1)	1.52 (0.76 – 3.01)	0.24

Supplementary Table S1: Patient characteristics for all cases of relapsed neuroblastoma, high risk cases, and intermediate risk, stage 3, unresectable, non-MNA cases.

Table 1:Patient characteristics for all cases of relapsed neuroblastoma, high risk cases and intermediate risk, stage 3, unresectable, non-MNA cases				
	All relapsed neuroblastoma N=189 N (%, 95% CI) [α]	High risk relapsed Neuroblastoma N=159 (84.1%) N (%, 95% CI) [α]	Intermediate risk (unresectable MYCN non-amplified) N=17 (9.0%) N (%, 95% CI) [β]	*P* – value[**] (high vs intermediate risk groups)
Gender				
Male	109 (57.7, 50.2-64.8)	92 (57.9, 49.8-65.6)	6 (35.3, 17.3-58.7)	0.08
Female	80 (42.3, 35.2-49.7)	67 (42.1, 34.4-50.2)	11 (64.7, 41.3-82.7)	
Age at diagnosis (years)				
Mean	4.1	4.1	5.5	
Median	2.96	3.0	3.9	0.09
Range	0 – 19	0 – 19	0-15	
Inter-quartile range	2.0 – 4.6	2.1 – 4.8	2.9 – 5.3	
≤ 1 year	N = 14	N = 3	N = 1	
Age groups				
<18 months	28 (14.8, 10.1-20.7)	17 (10.7, 6.4-16.6)	1 (5.9, 1.0-27.0)	
≥18 months	161 (85.2, 79.3-89.9)	142 (89.3, 83.4-93.6)	16 (94.1, 73.0-99..0)	0.52
Stage at diagnosis	N =185 (97.9)*	N=158 (99.4)*		
Stage 1, 2, 4s	5 (2.7, 0.9-6.2)	2 (1.3, 0.2-4.5)		
Stage 3	20 (10.8, 6.7-16.2)	3 (1.9, 0.4-5.4)		
Stage 4	160 (86.5, 80.7-91.1)	153 (98.1, 92.7-99.0)	-	-
Missing	4 (2.1)[b]	1 (0.6) [b]		
INPC histology[a]	N = 153 (81.0)*	N = 129 (81.1)*	N = 15 (88.2)*	
Unfavourable	144 (94.1, 89.1-97.3)	124 (96.1, 91.2-98.7)	13 (86.7, 62.1-96.2)	
Favourable	9 (5.9, 2.7-10.9)	5 (3.9, 1.3-8.8)	2 (13.3, 3.7-37.9)	0.16
Missing	36 (19.0) [b]	30 (18.9) [b]	2 (11.8)	
Site of primary tumour	N = 186 (98.4)*	N = 156 (98.1)*	N = 17 (100.0)	
Abdominal (includes adrenal and retroperitoneal)	167 (89.8, 84.5-93.7)	143 (91.6, 86.2-95.5)	14 (82.4, 59.0-93.8)	0.14
Thoracic, spinal and cervical	14 (7.5, 4.2-12.3)	9 (5.7, 2.7-10.7)	3 (17.7, 6.2-41.0)	
Other	5 (2.7, 0.8-6.2)	4 (2.6, 0.7-6.4)	0 (0.0)	
Missing	3 (1.6) [b]	3 (1.9) [b]	-	

Lymph node metastasis at diagnosis	N = 169 (89.4)	N =142 (89.3)	N = 17 (100)	
No Yes Missing	95 (56.2, 48.3-63.8) 74 (43.8, 36.2-51.6) 30 (10.6) [b]	74 (52.1, 43.6-60.6) 68 (47.9, 39.4-56.4) 17 (10.7) [b]	12 (70.6, 46.9-86.7) 5 (29.4, 13.3-53.1) -	0.15
Bone metastasis at diagnosis	N = 173 (91.5)*	N = 146 (91.8)	N = 17 (100)	
No Yes Missing	60 (34.5, 27.6-42.3) 114 (65.5, 58.3-72.9) 16 (8.5) [b]	36 (24.7, 17.9-32.5) 110 (75.3, 67.5-82.1) 13 (8.2) [b]	17 (100, --) 0 (0.0, --) -	-
Bone marrow metastasis at diagnosis	N = 173 (91.5)*	N = 146 (91.8)	N = 17 (100)	
No Yes Missing	51 (29.5, 22.8-36.9) 122 (70.5, 63.1-77.2) 16 (8.5)[b]	26 (17.8, 12.0-25.0) 120 (82.2, 75.0-88.0) 13 (8.2) [b]	17 (100.0, --) 0 (0.0, --) -	-
Liver metastasis at diagnosis	N = 166 (87.8)*	N = 166 (87.8)*	N = 17 (100)	
No Yes Missing	147 (88.5, 82.7-93.0) 19 (11.5, 7.0-17.3) 23 (12.2) [b]	122 (87.8, 66.1-80.0) 17 (12.2, 6.1-15.9) 20 (12.6) [b]	17 (100, --) 0 (0.0, --) -	-
Urinary catecholamine levels at diagnosis	N = 137 (72.5)*	N = 116 (73.0)*	N = 13 (76.5)*	
Normal Elevated Missing	7 (5.1, 2.1-10.2) 130 (68.8, 89.8-97.9) 52 (27.5) [b]	3 (2.6, 0.5-7.4) 113 (97.4, 92.6-99.5) 43 (27.0) [b]	2 (15.4, 4.3-42.2) 11 (84.6, 57.8-95.7) 4 (23.5) [b]	0.12
***MYCN* status**	N = 134 (70.9)*	N = 116 (73.0)*	N = 10 (58.8)*	
Amplified Not amplified, *MYCN* gain Not amplified Missing	49 (36.6, 28.4-45.3) 7 (5.2, 2.1-10.5) 78 (58.2, 49.4-66.7) 55 (29.1) [b]	49 (42.2, 33.1-51.8) 3 (2.6, 0.5-7.4) 64 (55.2, 45.7-64.4) 43 (27.0) [b]	- 2 (20.0, 5.7-51.0) 8 (80.0, 49.0-94.3) 7 (41.2) [b]	-
1p status	N = 65 (34.4)*	N= 57 (35.8)*	N=3 (15.0)*	
1p deleted 1p normal Missing	37 (56.9, 44.0-69.2) 28 (43.1, 30.8-56.0) 124 (65.6) [b]	35 (61.4, 47.6-74.0) 22 (38.6, 26.0-52.4) 102 (64.2) [b]	 3 (100.0, 43.9-100) 14 (85.0) [b]	-
***MYCN* and 1p**	N = 62 (32.8)*	N = 54 (34.0)*		
Non-MNA and 1p normal MNA and 1p normal Non-MNA and 1p deleted MNA and 1p deleted Missing	23 (37.1, 25.2-50.3) 3 (4.8, 1.0-13.5) 7 (11.3, 4.7-21.9) 29 (46.7, 34.0-60.0) 127 (67.2)	17 (31.5, 19.5-45.6) 3 (5.6, 1.2-15.4) 5 (9.3, 3.1-20.3) 29 (53.7, 39.6-67.4) 105 (66.0)	-	-
Year of diagnosis				

≤ 2000 >2000	100 (52.9, 45.5-60.2) 89 (47.14, 39.8-54.5)	82 (51.6, 43.5-59.6) 77 (48.4, 40.4-56.5)	10 (58.8, 41.3-82.7) 7 (41.2, 21.6-64.0)	0.57
Overall response at end of treatment	N = 166 (87.8)*	N = 139 (87.4)*	15 (88.2)*	
Stable disease Mixed response Partial response Complete response Progressive disease Missing	7 (4.2, 1.7-8.5) 12 (7.2, 3.8-12.3) 82 (49.4, 41.6-57.3) 40 (24.1, 17.8-31.3) 25 (15.1, 10.0-21.4) 23 (12.2)	5 (3.6, 1.2-8.2) 12 (8.6, 4.5-14.6) 70 (50.4, 41.8-58.9) 30 (21.6, 15.1-29.4) 22 (15.8, 10.2-23.0) 20 (12.6)	1 (6.7, 1.2-29.8) 0 (0.0, 0-20.4) 7 (46.7, 24.8-70.0) 6 (40.0, 19.8-64.3) 1 (6.7, 1.2-29.8) 2 (11.8)	0.36

[a] International Neuroblastoma Pathology Classification (Shimada *et al*, 1999)[49]

***** Percent of the total number of cases.

[**] Chi-squared test and the Fisher's exact test (where numbers were less than 5) were used to compare between high and intermediate risk group characteristics. [α] 95% CI were calculated for percentages using the asymptotic method.

[b] Percent with missing data. [β] 95% CI for proportions were calculated using the Wilson score method.

Supplementary Table S2: Treatment received at diagnosis for all relapsed neuroblastoma cases, high risk cases and for intermediate risk, unresectable, non-MNA neuroblastoma.

Supplementary Table S1: Treatment received at diagnosis for all relapsed neuroblastoma cases, high risk cases and for intermediate risk, unresectable, non-MNA neuroblastoma.									
Treatment	**All relapsed cases N (%)**			**High Risk N (%)**			**Intermediate Risk N (%)**		
	Diagnosed ≤ 2000	**Diagnosed > 2000**	**Total**	**Diagnosed ≤ 2000**	**Diagnosed > 2000**	**Total**	**Diagnosed ≤ 2000**	**Diagnosed > 2000**	**Total**
ENSG5 trial[a]	56 (58.9)	0 (0.0)	56 (30.4)	50 (64.9)	0 (0.0)	50 (32.5)	4 (40.0)	0 (0.0)	4 (23.5)
ENSG5 protocol	7 (7.4)	2 (2.2)	9 (4.9)	7 (9.1)	2 (2.6)	9 (5.8)	0 (0.0)	0 (0.0)	0 (0.0)
Kushner trial[b]	4 (4.2)	4 (4.5)	8 (4.3)	4 (5.2)	4 (5.2)	8 (5.2)	0 (0.0)	0 (0.0)	0 (0.0)
HRNBL1 trial[c]	0 (0.0)	56 (62.9)	56 (30.4)	0 (0.0)	53 (68.8)	53 (34.4)	0 (0.0)	2 (28.6)	2 (11.8)
HRNBL1 protocol	3 (3.2)	9 (10.1)	12 (6.5)	3 (3.9)	9 (11.7)	12 (7.8)	0 (0.0)	0 (0.0)	0 (0.0)
ENSG9[d]	2 (2.1)	2 (2.2)	4 (2.2)	n/a	n/a	n/a	2 (20.0)	2 (28.6)	4 (23.5)
Other trials*	10 (10.5)	5 (5.6)	15 (8.2)	6 (7.8)	3 (3.9)	9 (5.8)	1 (10.0)	1 (14.3)	2 (11.8)
Other treatments **	13 (13.7)	11 (12.4)	24 (13.0)	7 (9.1)	6 (7.8)	13 (8.4)	3 (30.0)	2 (28.6)	5 (29.4)
Total	95 (100) [α]	89 (100)	184 (100) [α]	77 (100)[α]	77 (100)	154 (100) [α]	10 (100)	7 (100)	17 (100)

[a] European Neuroblastoma Study group Fifth study (ENSG5) (Pearson *et al*, 2008)
[b] N7 Protocol (Kushner) (Kohler *et al*, 2007)
[c] European High Risk Neuroblastoma Study 1 of SIOP-Europe (HRNBL1) (Ladenstein *et al*, 2010)
[d] ENSG9 (ClinicalTrials.gov identifier: NCT00276731)
* Other trials include infant neuroblastoma study, MIBG2, or unknown. ** Other treatments were chemotherapy, chemotherapy and surgery, chemotherapy and surgery and radiotherapy, or chemotherapy and surgery and radiotherapy and SCT. Chemotherapy was usually OPEC/OJEC, CADO, topotecan, TVD, carboplatin-etoposide, cyclophosphamide, vincristine.
[α] Five cases diagnosed in 1990 had no available files.

Supplementary Table S3: Treatment received at 2nd and subsequent relapse for all relapsed neuroblastoma cases, high risk cases and for intermediate risk, unresectable, non-MNA neuroblastoma.

Supplementary Table S2: Treatment received at 2nd and subsequent relapse for all relapsed neuroblastoma cases, high risk cases and for intermediate risk, unresectable, non-MNA neuroblastoma.									
Treatment	**All relapsed cases N (%)**			**High Risk N (%)**			**Intermediate risk N (%)**		
	Diagnosed ≤ 2000	**Diagnosed > 2000**	**Total**	**Diagnosed ≤ 2000**	**Diagnosed > 2000**	**Total**	**Diagnosed ≤ 2000**	**Diagnosed > 2000**	**Total**
Second line Chemotherapy*	10 (34.5)	21 (42.0)	31 (39.2)	8 (34.8)	18 (43.9)	26 (40.6)	2 (100.0)	3 (50.0)	5 (62.5)
Combination of treatments**	3 (10.3)	6 (12.0)	9 (11.4)	3 (13.0)	3 (7.3)	7 (10.9)	0 (0.0)	1 (16.7)	1 (12.5)
mIBG Therapy	0 (0.0)	1(2.0)	1(1.3)	0 (0.0)	1 (2.4)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
Other (e.g. Surgery or radiotherapy)	1 (3.5)	5 (10.0)	6 (7.6)	1 (4.4)	3 (7.3)	4 (6.3)	0 (0.0)	1 (16.7)	1 (12.5)
Phase I or II trials	0 (0.0)	3 (6.0)	3 (3.8)	0 (0.0)	3 (7.3)	3 (4.7)	0 (0.0)	0 (0.0)	0 (0.0)
Palliative radiotherapy	15 (51.7)	10 (20.0)	25 (31.7)	0 (0.0)	4 (9.8)	4 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)
Supportive care	0 (0.0)	4 (8.0)	4 (5.1)	11 (47.8)	8 (19.5)	19 (29.7)	0 (0.0)	1 (16.7)	1 (12.5)
Total	**29 (100)**	**50 (100)**	**79 (100)**	**23 (100)**	**41 (100)**	**64 (100)**	**2 (100)**	**6 (100)**	**8 (100)**

* Second line chemotherapy includes vincristine, temozolamide, irinotecan, topotecan vincristine-doxorubicin or oral etoposide.
** Combination of treatments includme chemotherapy and surgery or radiotherapy.

Supplementary Table S4: Cox univariable analysis for post relapse overall survival time and relationship with clinical and biological variables for high risk cases

Supplementary Table S3: Cox univariable analysis for post relapse overall survival time and relationship with clinical and biological variables for high risk cases			
Factor	**N (%)**	**Hazard ratio (95% CI)**	***P*-value**
Age at diagnosis			
>18 months	142 (89.3)	1	
≤18 months	17 (10.7)	1.15 (0.67 – 1.97)	0.61
Age at diagnosis			
≤ 5 years	126 (79.3)	1	
> 5 years	33 (20.7)	0.62 (0.42 – 0.93)	0.02
Gender			
Males	92 (57.9)	1	
Females	67 (42.1)	1.14 (0.82 – 1.58)	0.42
Bone metastases at diagnosis			
Yes	110 (75.3)	1	
No	36 (24.7)	1.19 (0.80 – 1.76)	0.39
Bone marrow metastases at diagnosis			
Yes	120 (82.2)	1	
No	26 (17.8)	0.67 (0.42 – 1.06)	0.08
Liver metastases at diagnosis			
No	122 (87.8)	1	
Yes	17 (12.2)	1.95 (1.16 – 3.27)	0.02
Bone metastases at relapse			
No	70 (49.3)	1	
Yes	72 (50.7)	1.24 (0.88 – 1.74)	0.23
Bone marrow metastases at relapse			
No	58 (50.0)	1	
Yes	58 (50.0)	0.86 (0.59 – 1.26)	0.44
***MYCN* status**			
Not amplified	67 (57.8)	1	
Amplified	49 (42.2)	2.10 (1.42 – 3.10)	<0.001
1p status			
1p normal	22 (38.6)	1	
1p deleted	35 (61.4)	1.58 (0.90 – 2.78)	0.11
***MYCN* disease and 1p status**			
MYCN non amplified and 1p normal	17 (31.5)	1	
Either *MYCN* amplified or 1p deleted	8 (14.8)	1.71 (0.71 – 4.11)	0.23
MYCN amplified and 1p deleted	29 (53.7)	2.11 (1.11 – 4.01)	0.02
Time from diagnosis to relapse			
>24 months	35 (22.0)	1	
18 – 24 months	28 (17.9)	1.14 (0.68 – 1.92)	0.62
12 – 18 months	40 (25.2)	1.24 (0.77 – 2.00)	0.37
6 – 12 months	32 (20.1)	1.44 (0.87 – 2.38)	0.16
< 6 months	24 (15.1)	1.82 (1.06 – 3.14)	0.03

Supplementary Table S5: Profile of survivors at the end of the study period (N = 16).

Table 4: Profile of survivors at the end of the study period (N = 16)	
Characteristic	**N (%, 95% CI)**
Gender	
Male	10 (62.5, 38.6-81.5)
Female	6 (37.5, 18.5-61.4)
Age at diagnosis (years)	
Mean	2.9
Median	1.8
Range	0 – 12.8
≤ 1 year	N = 7
Age group	
<18 months	8 (50.0, 28.0-72.0)
≥18 months	8 (55.0, 28.0-72.0)
Risk Group	N = 15
High risk	5 (33.3, 15.2-58.3)
Intermediate unresectable stage 3 non-MNA	4 (26.6, 10.9-52.0)
Intemediate stage 4 non-MNA infants	4 (26.6, 10.9-52.0)
Low risk	2 (13.3, 3.7-37.9)
Site of primary at diagnosis	
Abdominal (includes adrenal and retroperitoneal)	13 (81.3, 57.0-93.4)
Thoracic and spinal	3 (18.7, 6.6-43.0)
***MYCN* status**	N = 13
Amplified	1 (7.7, 1.4-33.3)
Not amplified, *MYCN* gain	1 (7.7, 1.4-33.3)
Not amplified	11 (84.6, 57.8-95.7)
1p status	N = 6
1p deleted	2 (33.3, 9.7-70.0)
1p normal	4 (66.6, 30.0-90.3)
***MYCN* disease and 1p status**	N = 6
MYCN non amplified and 1p normal	4 (66.7, 30.0-90.3)
Either *MYCN* amplified or 1p deleted or both	2 (33.3, 9.7-70.0)
INPC histology	N = 15
Unfavourable	11 (73.3, 48.0-89.1)
Favourable	4 (26.7, 10.9-51.6)
Year of diagnosis	
≤ 2000	8 (50.0, 28.0-72.0)
>2000	8 (50.0, 28.0-72.0)
Overall response at end of treatment	N = 15
Stable disease	1 (6.7, 1.2-29.8)
Mixed response	0 (0.0, 0-20.4)
Partial response	6 (40.0, 19.8-64.3)
Complete response	8 (53.3, 30.1-75.2)
Progressive disease	0 (0.0, 0-20.4)
Time to relapse	
Mean	22.4 months
Median	23.0 months
Inter-quartile range	8.1 – 35.4 months
Range	1.5 – 48.9 months
Time from diagnosis to relapse	
< 6 months	4 (25.0, 10.2-49.5)
6 – 18 months	2 (12.5, 3.5-36.0)
18 – 24 months	2 (12.5, 3.5-36.0)
>24 months	8 (50.0, 28.0-72.0)

* 95 % CI for proportions calculated using the Wilson score method.

Supplementary Table S6: Comparison of clinical characteristics of survivors with non-survivors of relapsed neuroblastoma.

Supplementary Table S4: Comparison of clinical characteristics of survivors with non-survivors of relapsed neuroblastoma.			
Characteristic	Non-Survivors N = 173 (%, 95% CI) [α]	Survivors N = 16 (%, 95% CI) [β]	P-value
Gender			
Male	99 (57.2, 49.5-64.7)	10 (62.5, 38.6-81.5)	
Female	74 (42.8, 35.3-50.5)	6 (37.5, 18.5-61.4)	0.45
Age at diagnosis (years)			
Mean	4.2	2.9	
Median	3.1	1.8	
Range	0-19.5	0 – 12.8	0.02*
≤ 1 year	N=7	N = 7	
Age group			
<18 months	20 (11.6, 7.2-17.3)	8 (50.0, 28.0-72.0)	
≥18 months	153 (88.4, 82.7-92.8)	8 (55.0, 28.0-72.0)	<0.001
INPC histology	**N = 138**	**N = 15**	
Unfavourable	133 (96.4, 91.7-98.8)	11 (73.3, 48.0-89.1)	
Favourable	5 (3.6, 1.2-8.3)	4 (26.7, 10.9-51.6)	0.006
Time to relapse			
Mean	17.7 months	22.4 months	
Median	14.37 months	23.0 months	0.14
Inter-quartile range	9.2 – 21.0 months	8.1 – 35.4 months	
Range	1.3 – 96.2 months	1.5 – 48.9 months	
Time from diagnosis to relapse			
< 6 months	28 (16.2, 11.0-22.5)	4 (25.0, 10.2-49.5)	
6 – 18 months	79 (45.7, 38.1-53.4)	2 (12.5, 3.5-36.0)	0.01
18 – 24 months	30 (17.3, 12.0-23.8)	2 (12.5, 3.5-36.0)	
>24 months	36 (20.8, 15.0-27.6)	8 (50.0, 28.0-72.0)	
Overall response at end of 1st line treatment	N = 151	N = 15	
Stable disease	6 (4.0, 1.3-7.4)	1 (6.7, 1.2-29.8)	
Mixed response	12 (8.0, 3.6-11.8)	0 (0.0, 0-20.4)	
Partial response	76 (50.3, 36.4-51.7)	6 (40.0, 19.8-64.3)	0.04
Complete response	32 (21.2, 13.0-25.1)	8 (53.3, 30.1-75.2)	
Progressive disease	25 (16.6, 9.6-20.6)	0 (0.0, 0-20.4)	

* Comparison of medians of the 2 groups using the two-sample Wilcoxon rank-sum (Mann-Whitney) test. . [α] 95% CI were calculated for percentages using the asymptotic method. [β] 95% CI for proportions were calculated using the Wilson score method.

Figure 1: Flow diagram showing numbers of cases included in the study.

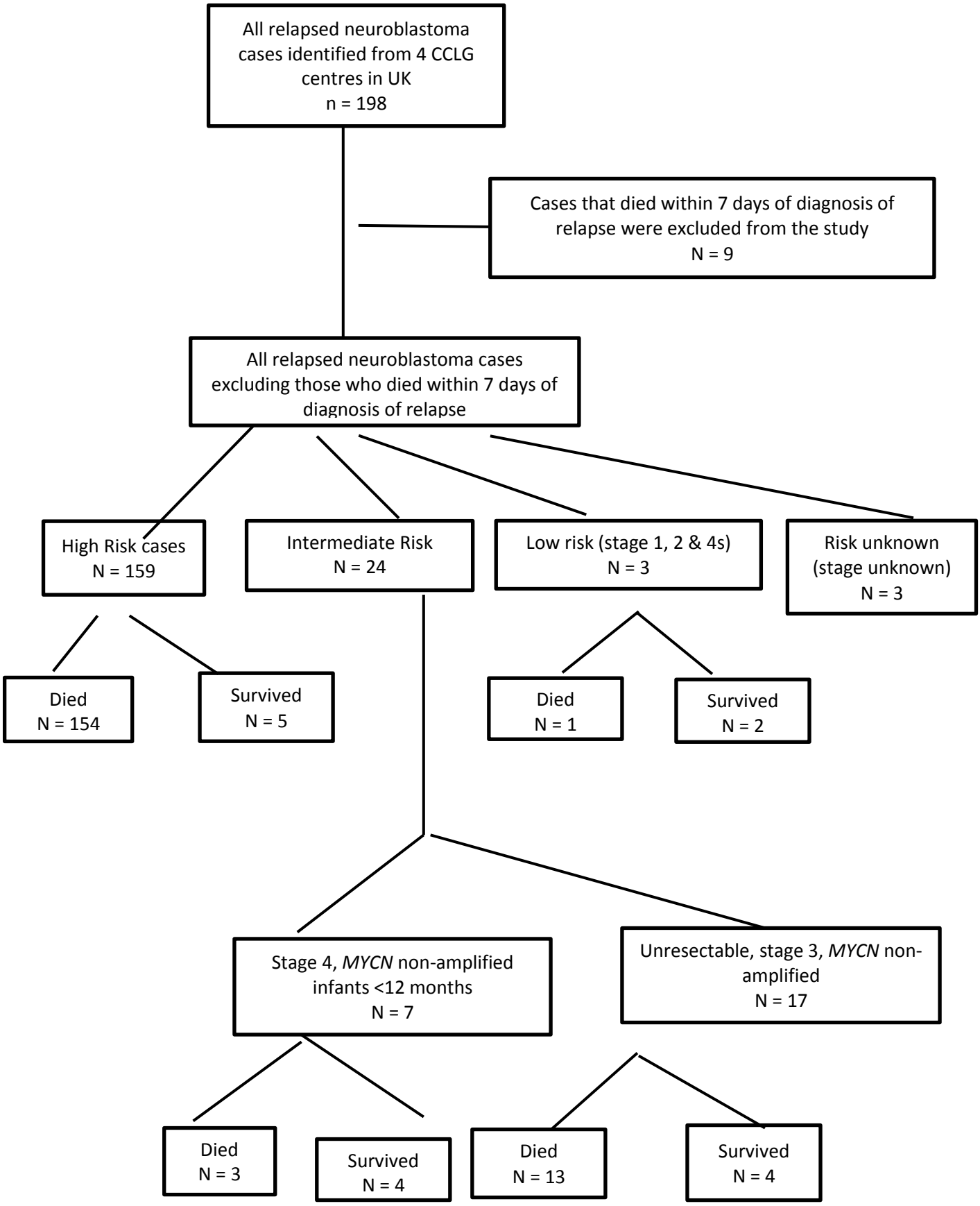
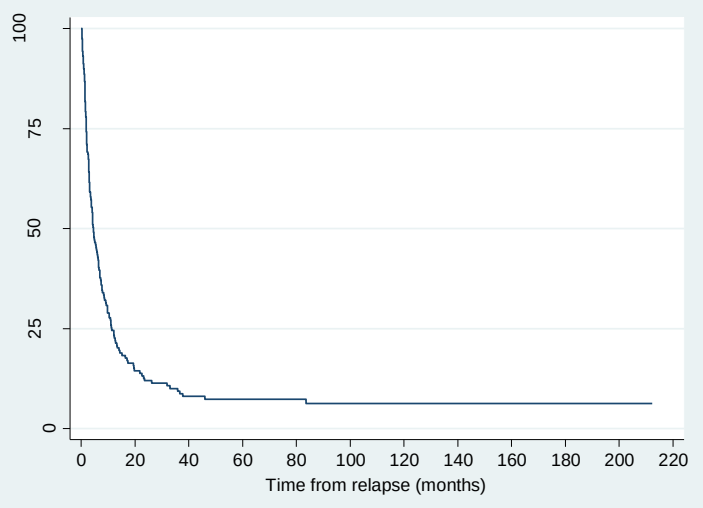


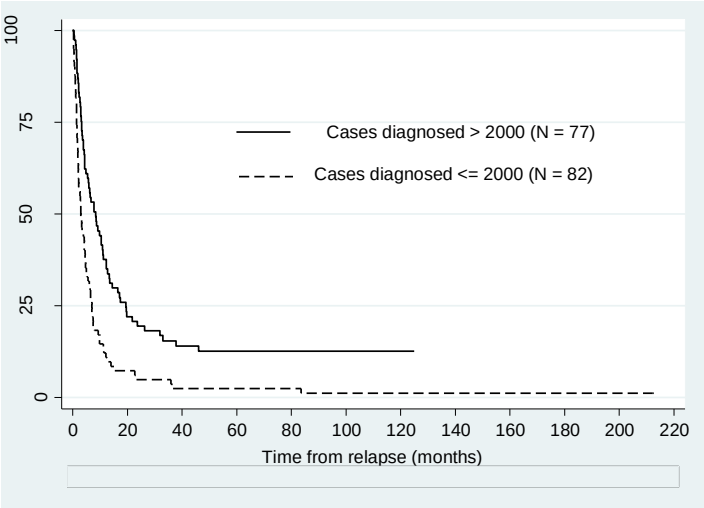
Figure 2: Kaplan Meier graphs for post relapse overall survival time for the high risk group (N = 159).

Figure 2a: Post relapse overall survival (PROS) time



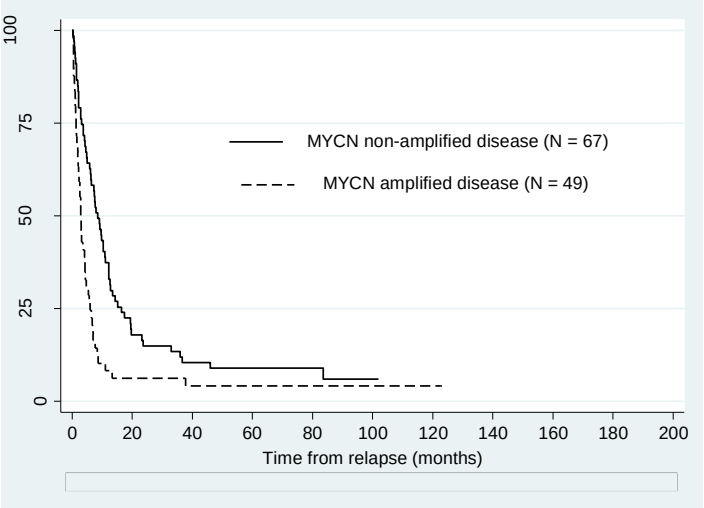
Median PROS time for high risk cases was 4.5 months (IQR = 1.9 – 11.4). 5 year PROS for high risk cases was 7.4% (95% CI 4.0-12.1%).

Figure 2b: Post relapse overall survival by year of diagnosis



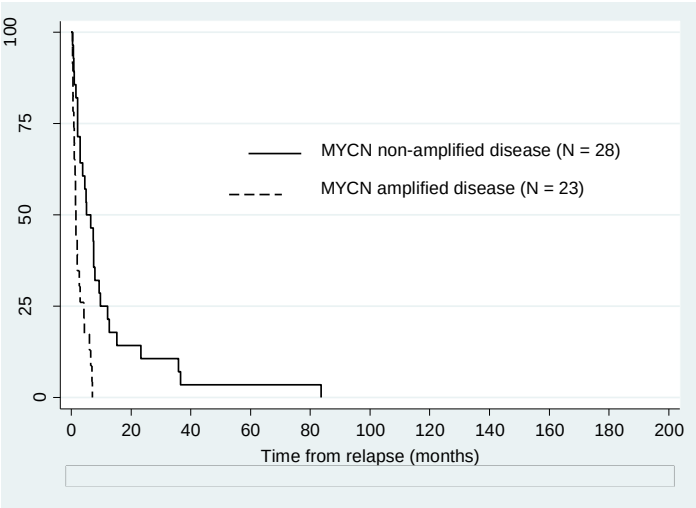
Median PROS time was 2.9 months (IQR 1.4 – 6.9) for cases diagnosed ≤ 2000 versus 8.4 months (IQR 3.0 – 17.4) for cases diagnosed > 2000 (*P* <0.001). 5 year PROS for high risk cases diagnosed ≤ 2000 was 2.4% (95% CI 0.5-7.7%) versus 12.7% (95% CI 6.4-21.2%) for cases diagnosed >2000.

Figure 2c: PROS by *MYCN* status



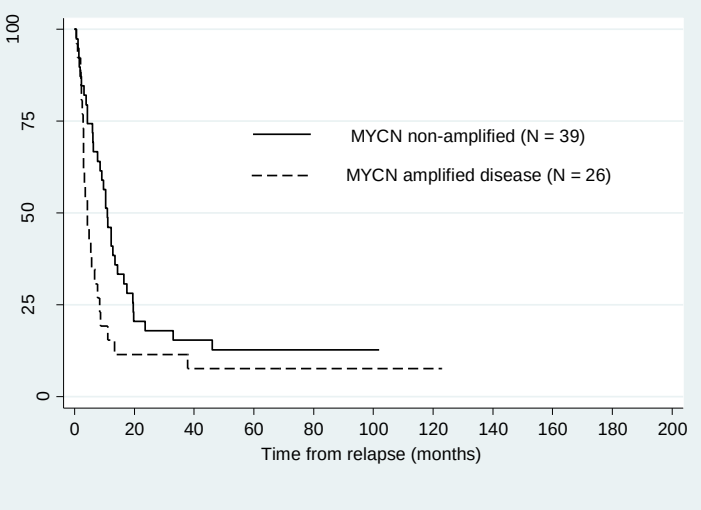
Median PROS time was 2.9 months (95% CI 2.0 – 4.3) for *MYCN* amplified versus 8.5 months for MYCN non-amplified (95% CI 5.9 –11.1) (*P* < 0.001).). 5 year PROS for *MYCN* non-amplified cases was 9.0% (95% CI 3.7-17.2%) versus 4.1% (95% CI 0.8-12.3%) for *MYCN* amplified cases.j

Figure 2d: PROS for cases diagnosed ≤ 2000 by *MYCN* status



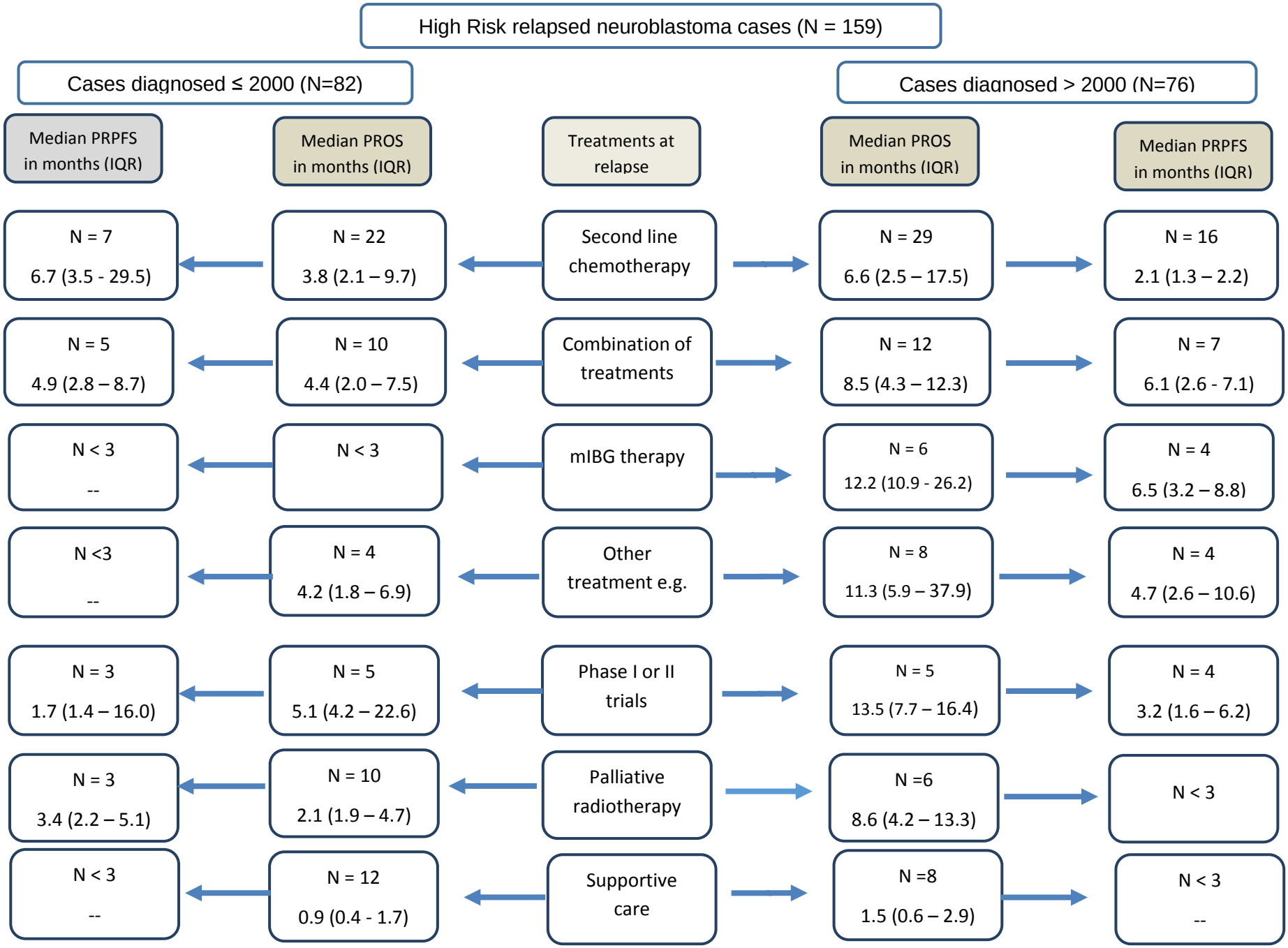
For cases diagnosed ≤ 2000, the median PROS for *MYCN* amplified disease was 1.5 months (95% CI 0.9 – 2.7) versus 5.1 months (95% CI 2.9 – 7.9) for *MYCN* non-amplified (*P* < 0.001). 5 year PROS for *MYCN* non-amplified cases was 3.6% (95% CI 0.3-15.4%) versus 0% for *MYCN* amplified cases.

Figure 2e: PROS for cases diagnosed > 2000 by *MYCN* status



For cases diagnosed > 2000, the median PROS for *MYCN* amplified disease was 4.3 months (95% CI 2.9 – 6.6) versus 10.9 months (95% CI 6.3 – 14.4) for *MYCN* non-amplified (*P*= 0.02). 5 year PROS for *MYCN* non-amplified cases was 12.8% (95% CI 4.7-25.2%) versus 7.7% (95% CI 1.3-21.7%) for *MYCN* amplified cases.

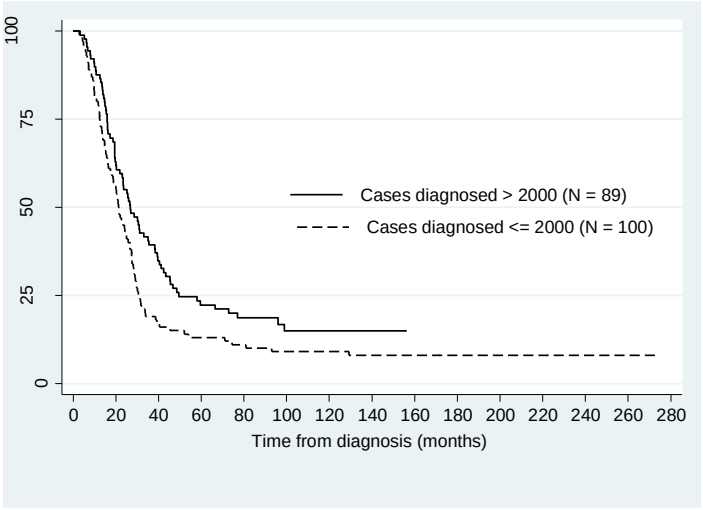
Figure 3: Flow diagram showing treatments high risk patients received at first relapse and its outcome.



PROS is post relapse overall survival. IQR is interquartile range.
PRPFS is post relapse progression free survival.

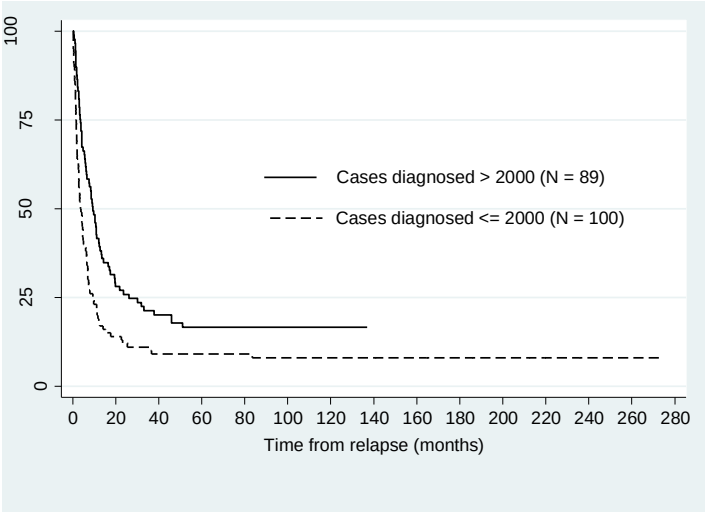
Supplementary Figure S1: Kaplan Meier graphs for overall survival time and post relapse overall survival time for all relapsed neuroblastoma cases by year of diagnosis (N=189).

Figure S1a: Overall survival (OS) time from diagnosis by year of diagnosis



Median OS time was 21.3 months (IQR 12.4 – 30.6 months) for cases diagnosed ≤ 2000 versus 26.8 months (IQR 15.9 – 49.5) for cases diagnosed > 2000 (*P* = 0.02). 5 year OS time was 13% (95% CI 7–20) for cases diagnosed ≤ 2000. 5 year OS time was 22% (95% CI 14 - 31) for cases diagnosed >2000.

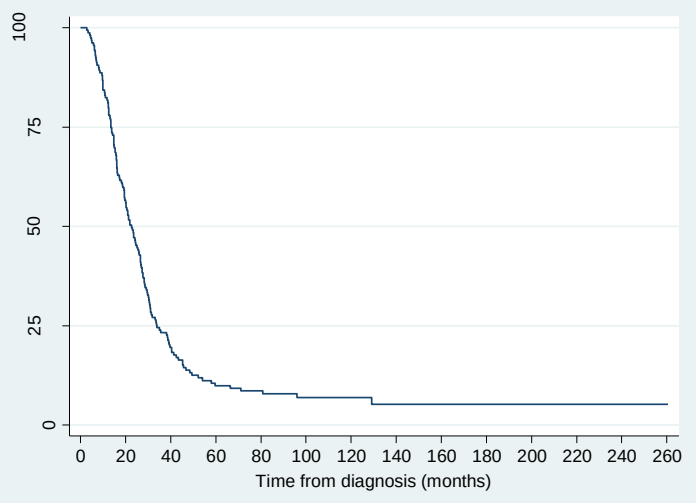
Figure S1b: Post relapse overall survival (PROS) by year of diagnosis.



Median PROS time was 3.5 months (IQR 1.5 – 9.3 months) for cases diagnosed ≤ 2000 versus 9.4 months (IQR 3.5 – 26.2) for cases diagnosed > 2000 (*P* = 0.001). 5 year PROS time was 9% (95% CI 4 - 16) for cases diagnosed ≤ 2000. 5 year PROS time was 17% (95% CI 10 - 25) for cases diagnosed >2000.

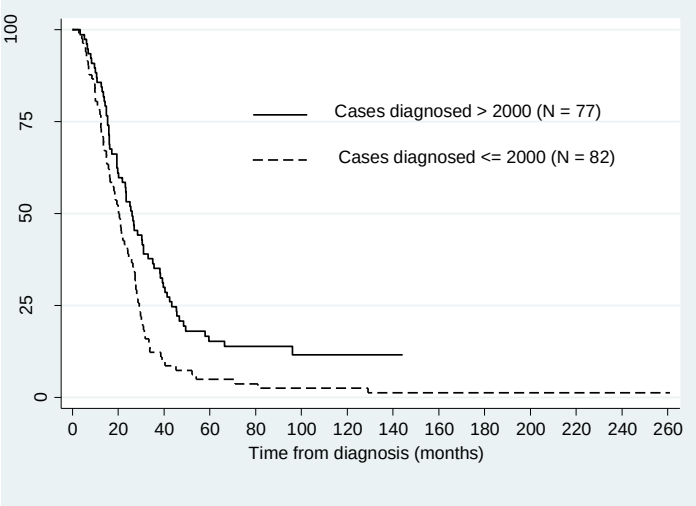
Supplementary Figure S2: Kaplan Meier graphs for overall survival time for the high risk group (N = 159).

Figure S2a: Overall survival (OS) time from diagnosis



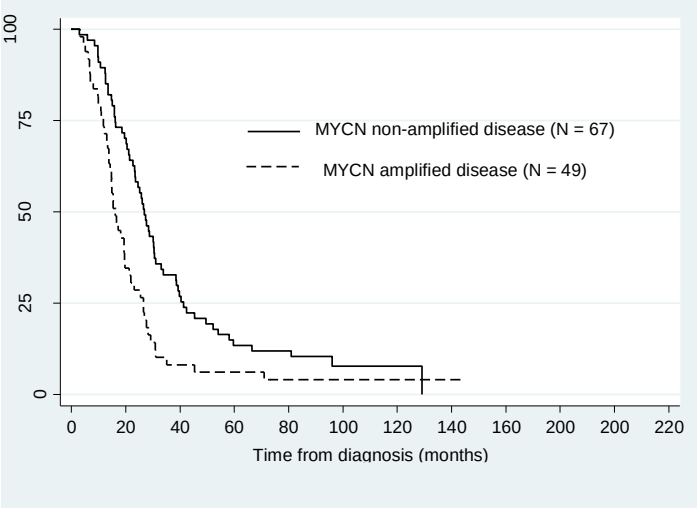
Median OS time for high risk cases was 22.7 months (inter-quartile range 13.5 – 33.8 months). 5 year OS 9.9% (95% CI 5.9-15.2%).

Figure S2b: Overall survival time by year of diagnosis



Median OS time was 20.2 months (IQR 12.4 – 29.2 months) for cases diagnosed ≤ 2000 versus 26.1 months (IQR 15.4 – 43.4) for cases diagnosed > 2000 (*P* = 0.001). 5 year OS for high risk cases diagnosed ≤ 2000 was 4.9% (95% CI 1.6-11.1%) versus 15.2% (95% CI 8.2-24.2%) for cases diagnosed >2000.

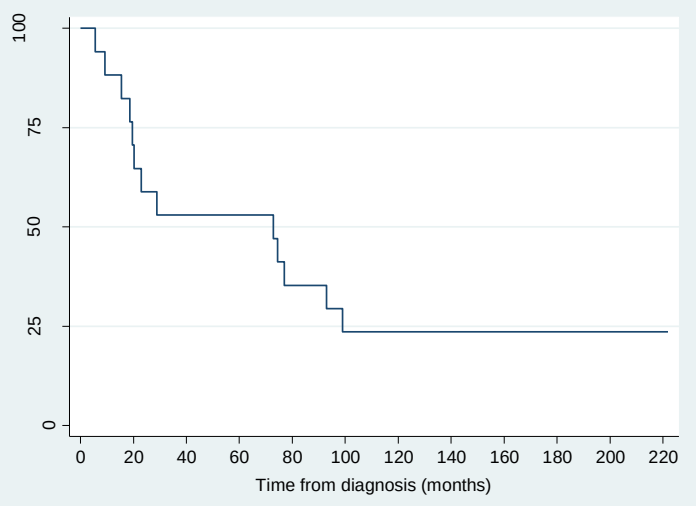
Figure S2c: Overall survival time by *MYCN* status



Median OS time was 16.1 months (95% CI 13.9 – 19.5 months) for *MYCN* amplified versus 26.8 months (95% CI 22.7 – 30.6 months) for non-*MYCN* amplified (*P* = 0.001). 5 year PROS for *MYCN* non-amplified cases was 13.4% (95% CI 6.6-22.7%) versus 6.1% (95% CI 1.6-15.2%) for *MYCN* amplified cases.

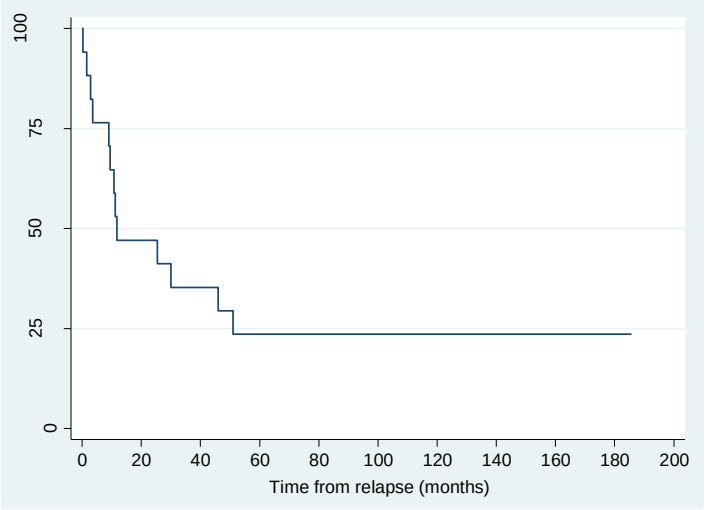
Supplementary Figure S3: Kaplan Meier graphs for overall survival time and post relapse overall survival time for the intermediate risk, unresectable non-MNA risk group (N = 17).

Figure S3a: Overall survival time from diagnosis



Median OS time for the intermediate, stage 3 unresectable *MYCN* non-amplified cases was 72.9 months (inter-quartile range 19.5 – 99.0 months). The 5 year OS was 53% (95% CI 28 - 73).

Figure S3b: Post relapse overall survival time



Median PROS time for the intermediate, stage 3 unresectable *MYCN* non-amplified cases was 11.8 months (inter-quartile range 9.0 – 51.6 months). The 5 year PROS was 24% (95% CI 7 - 45).